Structural Insights into Conformational Changes Required for Kinesin Motility

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Introduction: Kinesin motors transport organelles, vesicles and possibly chromosomes on microtubules using chemical energy from ATP hydrolysis to generate force. The kinesin motor ATPase is activated by microtubule binding, which enables motor movement on microtubules. However, the structural basis for motor ATPase activation is not known. We made three mutants of Kar3, a kinesin homologue from yeast, that completely block the microtubule-stimulated ATPase activity of the motor while these mutants still maintained microtubule binding affinity as good as wild-type Kar3.

Methods and Materials: We determined the structures of a wild-type motor domain protein, Kar3+N11, to 1.5Å resolution and three Kar3 mutants that uncouple microtubule- and nucleotide-binding by the motor or disable a salt bridge between the conserved 'switch I' and 'switch II' regions of the motor to 1.7Å, 1.3Å and 2.5Å resolutions, respectively. Diffraction data were collected from crystals grown at 18°C (Kar3+N11 and the uncoupling mutant, NK) or 4°C (the switch I and II salt-bridge mutants) at the beamline X4A. The structure of Kar3+N11 was determined by refining the previous model of the Kar3 motor domain using the Kar3+N11 data. The structures of the NK uncoupling and the switch I salt-bridge mutants were determined by refining the final model of Kar3+N11. The structure of the switch II salt-bridge mutant was determined by molecular replacement using the refined Kar3+N11 as a search model.

Results: The structure of Kar3+N11, compared to the previous wild-type structure, identifies a helix near switch I to be inherently flexible. The structure of the NK uncoupling mutant shows that the single residue change in the mutant eliminates its interaction with the switch II loop. The structure of the switch I salt-bridge mutant shows striking conformational differences from wild-type Kar3. The differences include disordering of the region-surrounding switch I and stabilization of the switch II loop/helix. In the meantime, the structure of the switch II salt-bridge mutant shows less conformational differences from wild-type Kar3 that include disordered switch I loop.

Conclusions: The observed structural changes define a signaling pathway between the microtubule binding and the active site. The Kar3 mutants structures also provide a molecular mechanism for activation of the motor ATPase by microtubules, which involves the melting of a flexible helix near switch I.